PATENT COOPERATION TREATY

PCT CORRECTED VERSION

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference P035884WO	FOR FURTHER ACTION	See Form PCT/IPEA/416
International application No. PCT/GB2004/004739	International filing date *(day/month/year)* 10.11.2004	Priority date *(day/month/year)* 10.11.2003
International Patent Classification (IPC) or national classification and IPC C12N15/82		
Applicant UNIVERSITY OF KENT et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
01.09.2005	06.12.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Young, C Telephone No. +49 89 2399-7877

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004739

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-12	as originally filed
Claims, Numbers	
1-18	filed with the demand
Drawings, Sheets	
1-3	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18
	No:	Claims	
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-18
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents/:

D1: HENTZER MORTEN ET AL: "Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections." JOURNAL OF CLINICAL INVESTIGATION, vol. 112, no. 9, November 2003 (2003-11), pages 1300-1307, XP002316251 ISSN: 0021-9738

D2: ZHU JUN ET AL: "The quorum-sensing transcriptional regulator TraR requires its cognate signaling ligand for protein folding, protease resistance, and dimerization" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 98, no. 4, 13 February 2001 (2001-02-13), pages 1507-1512, XP002316250 ISSN: 0027-8424

D3: WILLIAMS PAUL ET AL: "Quorum sensing and the population-dependent control of virulence" PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY OF LONDON B BIOLOGICAL SCIENCES, vol. 355, no. 1397, 29 May 2000 (2000-05-29), pages 667-680, XP002316249 ISSN: 0962-8436

D4: RAMAGE GORDON ET AL: "Inhibition of Candida albicans biofilm formation by farnesol, a quorum-sensing molecule." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 68, no. 11, November 2002 (2002-11), pages 5459-5463, XP002316252 ISSN: 0099-2240

D1 to D4 disclose numerous mechanisms involved in the regulation of quorum sensing, comprising modulating the ability of LuxR or a homologue of LuxR to activate transcription. Moreover many of the homologues of claim 2 are explicitly disclosed in this cited prior art. However, no mention is made specifically to a method for use to regulate quorum sensing whereby the step of proteolysis is employed. As such the claims are regarded as being novel and therefore meeting the requirements of Article 33 (2) PCT.

Inventive step Article 33 (3) PCT

D1 is considered to be the closest prior art. D1 describes the pharmacological inhibition of quorum sensing components *inter alia* LuxR homologues. In particular specific blockers of receptors in the form of antagonists are mentioned, see page 1304 of D1. No mention is made of the use of peptide hydrolases.
The objective problem is defined as;
" the provision of an alternative means to up and down regulate quorum sensing in bacteria"

the solution to the problem being the abolition of receptor function by peptidase hydrolysis or the upregulation by peptidase inhibition.

it is clear from D1 that pharmacological quorum regulation is of importance.
The skilled person knows from D1 that the LuxR is intracellular and responds to external cues by diffusible signals transported across the plasma membrane, see figure 1 of D1.

Given the incentive and clear need to provide a means to abrogate quorum signalling, see D1, the skilled person would apply protease in an attempt to destroy extracellular signalling pathways.

The general use of non specific proteolysis as claimed would be expected to abrogate a biological event reliant on receptor signalling (see figure 1 of D1) and is self-evident to the person skilled in the art.

The claims do not read specific proteolysis of Lux R. Furthermore even if the claims were to be worded as such they still would relate to **up**- and down- regulation of quorum sensing. Just how this is possible given that the application only describes down regulation, presumably due to loss of function by proteolysis, is beyond what can be assumed credible.
As a consequence as there is no data supporting that the entire claimed scope is solved inventive step can not be recognized.

For this reason claims 1-18 can not be regarded as meeting Article 33 (3) PCT.

CLAIMS

1. A method of regulating quorum sensing comprising modulating the ability of LuxR or a homologue of LuxR to activate transcription, wherein quorum sensing is either i) downregulated by treating the bacteria with a peptide hydrolase or ii) upregulated by treating with a peptide hydrolase inhibitor.

2. A method according to claim 1 wherein said homologue of LuxR is selected from the list consisting of AhlR, AhyR, AsaR, BafR, BisR, BpsR, BviR, CarR, CepR, CerR, CinR, CsaR, CviR, EagR, EcbR, EchR, EsaR, ExpR, HalR, LasR, Mll8752, MupR, PcoR, PhzR, PmlR, PpuR, PsmR, PsyR, RaiR, RhiR, RhlR, SdiA, SdiR, SmaR, SolR, SpnR, SprR, SwrR, TraR, TriR, TrlR, TrnR, VanR, VsmR, Y4qH, YenR, YpeR, YpsR, YruR, YtbR and YukR.

3. A method according to claim 2 wherein said peptide hydrolase is selected from the group consisting of Arg-C proteinase, Asp-N endopeptidase, BNPS Skatole, CNBr, chymotrypsin, clostripain, formic acid, glutamyl endopeptidase, iodosobenzoic acid, lysC, NTCB (2-nitro-5-thiocyanobenzoic acid), pepsin, proline-endopeptidase, proteinase K, Staphylococcal peptidase I, thermolysin and trypsin.

4. A method according to claim 3 wherein biofilm formation on a surface is inhibited.

5. A method according to claim 4 wherein said biofilm is caused by *Pseudomonas*, *Burkholderia*, *Klebsiella*, *Acinetobacter*, *Flavobacterium*, *Enterobacter* or *Aerobacter*.

6. A method according to claim 4 or claim 5 wherein said surface is wood, glass, concrete, plastic, ceramic, porcelain or metal.

7. A method according to any one of claims 4 to 6 wherein said surface forms part of a denture, a contact lens, an artificial valve, a prosthetic implant, a catheter, a pacemaker or a surgical pin.

8. Use of a composition comprising a peptide hydrolase and an aqueous or a non-aqueous carrier for disrupting the quorum sensing signal pathway of bacteria.

9. A use according to claim 8, wherein the composition further comprises one or more compounds selected from the group consisting of a detergent, a surfactant, a biocide, a fungicide, an antibiotic or a mixture thereof.

10. A use according to claim 8 or claim 9 wherein the composition further comprises one or more of a pH regulator, a perfume, a dye or a colorant.

11. A use according to any one of claims 8 to 10, wherein said composition is in the form of a spray, a foam, a slurry, a dispensable liquid or is freeze dried

12. A method according to claim 1 or claim 2 wherein said peptide hydrolase inhibitor is selected from the group consisting of serine protease inhibitors, including PMSF and Benzamide; cysteine (thiol) protease inhibitors, including PHMB and leupeptin; aspartate (acidic) protease inhibitors, including pepstatin and DAN; and metalloprotease inhibitors, including EDTA and EGTA.

13. A method according to claim 12 wherein said bacteria is *Vibrio salmonicida, Aeromonas hydrophila, Burkholderia ambifaria, Burkholderia pseudomallei, Burkholderia mallei, Burkholderia stabilis, Burkholderia vietnamiensis, Burkholderia multivorans, Escherichia coli, Serratia marcescens, Salmonella typhi, Brucella suis, Brucella melitensis, Yersinia ruckeri, Hafnia alvei, Shigella flexneri, Serratia liquefaciens, Enterococcus faecalis, Pseudomonas aeruginosa, Burkholderia cepacia, Pseudomonas fluorescens, Providencia stuartii, Klebsiella aerogenes, Yersinia pestis, Yersinia enterocolitica* or *Yersinia pseudotuberculosis*.

14. A method according to claim 12 or claim 13 wherein an exogenous gene is inserted into the operon controlled by quorum sensing.

15. A method according to claim 14 wherein said exogenous gene is required to be transported to the bacterial cell surface.

16. A method according to claim 14 wherein said exogenous gene encodes an antigen.

17. A method according to claim 16 wherein said antigen is of bacterial or viral origin.

18. Use of a composition comprising a peptide hydrolase inhibitor and an aqueous or a non-aqueous carrier for upregulating the quorum sensing signal pathway of bacteria.